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Reviewer: Anne Corrigan

Timestamp: Sun Sep 09 16:32:22 EDT 2007

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Application No: 10523729 Version No: 2.0

**Input Set:****Output Set:**

**Started:** 2007-08-27 15:04:48.964  
**Finished:** 2007-08-27 15:04:50.086  
**Elapsed:** 0 hr(s) 0 min(s) 1 sec(s) 122 ms  
**Total Warnings:** 35  
**Total Errors:** 0  
**No. of SeqIDs Defined:** 50  
**Actual SeqID Count:** 50

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W 213	Artificial or Unknown found in <213> in SEQ ID (20)
W 213	Artificial or Unknown found in <213> in SEQ ID (21)
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Error code

Error Description

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<110> Hamoen, Leendert W  
Kuipers, Oscar Paul  
Lindner, Johanna Cordula

<120> Novel Bacterial Expression System

<130> I-2002.012 US

<140> 10523729

<141> 2007-08-27

<150> PCT/EP2003/008506

<151> 2003-08-01

<150> EP 02078248.8

<151> 2002-08-07

<160> 50

<170> PatentIn version 3.3

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<211> 29

<212> DNA

<213> Bacillus subtilis

<400> 1

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<210> 2

<211> 94

<212> DNA

<213> Bacillus subtilis

<400> 2

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ttgcccatta tgggcccgtt agtccaaaag gagg 94

<210> 3

<211> 113

<212> DNA

<213> Bacillus subtilis

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ttgctccttg cccattatgg gccgcttagt ccaaaaggag gtgcaaacag atg 113

<210> 4

<211> 20

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<213> Bacillus subtilis

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<220>  
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<223> n is a, t, g or c

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<222> (20)..(20)  
<223> n is a, t, g or c

<400> 4  
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<210> 5  
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<213> Bacillus subtilis

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<223> n is a, g, t or c

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<221> misc\_feature

<222> (33)..(33)

<223> n is a, g, t or c

<400> 5  
ttattnattt nnnatttaatt ntatannatn nan 33

<210> 6  
<211> 33  
<212> DNA  
<213> Bacillus subtilis

<400> 6  
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<210> 7  
<211> 43  
<212> DNA  
<213> Bacillus subtilis

<400> 7  
aaaagtacat atttcttcaa aggaaaaaag caaaagatgt ttt 43

<210> 8  
<211> 154  
<212> DNA  
<213> Bacillus subtilis

<400> 8  
aaaagtacat atttcttcaa aggaaaaaag caaaagatgt ttttagctga aggaaaaatg 60  
aaaacgaaag ataaaaacag aggctgaaag ccatttttaa gcgtttttct tttcttggtg 120  
catcatttac aatacatata accgcaagga gagg 154

<210> 9  
<211> 166  
<212> DNA  
<213> Bacillus subtilis

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aaaacgaaag ataaaaacag aggctgaaag ccatttttaa gcgtttttct tttcttggtg 120  
catcatttac aatacatata accgcaagga gaggaggaat cgcattg 166

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<400> 10	
ggtataatat	10
<210> 11	
<211> 10	
<212> DNA	
<213> Bacillus subtilis	
<400> 11	
agttgtaaag	10
<210> 12	
<211> 13	
<212> DNA	
<213> Bacillus subtilis	
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aaaannnnnt ttt	13
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<211> 13	
<212> DNA	
<213> Bacillus subtilis	
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<221> misc_feature	
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<223> n is a, g, t or c	
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<211> 13	
<212> DNA	
<213> Bacillus subtilis	
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<221> misc_feature	
<222> (5)..(9)	
<223> n is a, t, g or c	

<400> 14  
 aatannnnnt att 13

<210> 15  
 <211> 13  
 <212> DNA  
 <213> Bacillus subtilis

<220>  
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 <222> (5)..(9)  
 <223> n is a, g, t or c

<400> 15  
 aaaannnnnt att 13

<210> 16  
 <211> 30  
 <212> DNA  
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<220>  
 <223> forward primer to amplify the ssb promoter region

<400> 16  
 gcgaagcttc caaacattga cgaagagtct 30

<210> 17  
 <211> 31  
 <212> DNA  
 <213> unknown

<220>  
 <223> Reverse primer to amplify the ssb promoter region

<400> 17  
 gctggatcct cggttaagca taagaaagac c 31

<210> 18  
 <211> 315  
 <212> DNA  
 <213> unknown

<220>  
 <223> PCR amplification product using Bacillus subtilis as template  
 source and SEQ ID NO: 17 and SEQ ID NO:18 as primers

<400> 18  
 gcttccaaac attgacgaag agtctaaaaa agcagttatc gagcgtttca acaacgttct 60

gacttctaac ggtgcggaag tcaactggaac aaaggattgg ggtaaagcgc gtcttgctta 120



cgaaatcaac gatttccgcg acggtttcta ccaaatcgta aacgttcaat ctgacgctgc	180
ggcagttcaa gaatttgacc gtctagctaa gatcagtgac gatatcattc gccacattgt	240
tgttaaagaa gaagaataag caattttgaa atatataatg gtaaaagggtg gtctttctta	300
tgcttaaccg aggat	315

<210> 19  
 <211> 28  
 <212> DNA  
 <213> unknown

<220>  
 <223> Forward primer to amplify the rpsF promoter region

<400> 19	
gcgaagcttg tgactttgag cggggcctt	28

<210> 20  
 <211> 622  
 <212> DNA  
 <213> unknown

<220>  
 <223> PCR amplification product using Bacillus subtilis as template  
 source and SEQ ID NO:18 and SEQ ID NO:20 as primers

<400> 20	
gcttgtgact ttgagcgggg cttcattcgt gctgagacag ttgcttatga ggatcttctt	60
gcgggcggcg gtatggcagg agctaaagag gcaggaaaag tccgccttga agggaaagaa	120
tatgtggtcc aagacggaga tgttattcat ttccgattta atgtatagga tgcagttgta	180
aagggacaag agctttggta taatataaaa ttgtgagtaa tagaattatt gtccttgcc	240
cattatgggc cgcttagtcc aaaaggaggt gcaaacagat gagaaagtac gaagttatgt	300
acattatccg cccaaacatt gacgaagagt ctaaaaaagc agttatcgag cgtttcaaca	360
acgttctgac ttctaacggt gcggagatca ctggaacaaa ggattggggg aaacgtcgtc	420
ttgcttacga aatcaacgat ttccgcgacg gtttctacca aatcgtaaac gttcaatctg	480
acgctgcggc agttcaagaa ttgaccgtc tagctaagat cagtgacgat atcattcgcc	540
acattgttgt taaagaagaa gaataagcaa ttttgaaata tataatggta aaaggtggtc	600
tttcttatgc ttaaccgagg at	622

<210> 21  
 <211> 30  
 <212> DNA

<213> unknown

<220>

<223> Reverse primer to amplify the rpsF promoter region

<400> 21

gctggatcca tcttcgtcaa tgtttgggcg 30

<210> 22

<211> 331

<212> DNA

<213> unknown

<220>

<223> PCR amplification product using Bacillus subtilis as template  
source and SEQ ID NO:20 and SEQ ID NO:22 as primers

<400> 22

gcttgtgact ttgagcgggg cttcattcgt gctgagacag ttgcttatga ggatcttctt 60

gcgggcgggcg gtatggcagg agctaaagag gcaggaaaag tccgccttga agggaaagaa 120

tatgtggtcc aagacggaga tgttattcat ttccgattta atgtatagga tgcagttgta 180

aagggacaag agctttggta taatataaaa ttgtgagtaa tagaattatt gtccttgcc 240

cattatgggc cgcttagtcc aaaaggaggt gcaaacagat gagaaagtac gaagttatgt 300

acattatccg cccaacatt gacgaagatg g 331

<210> 23

<211> 26

<212> DNA

<213> unknown

<220>

<223> Forward primer to amplify the ywpH promoter region

<400> 23

cccaagcttt caagctgtca atgccg 26

<210> 24

<211> 27

<212> DNA

<213> unknown

<220>

<223> Reverse primer to amplify the ywpH promoter region

<400> 24

cgcggatccg attgaacatg cgattcc 27

<210> 25

<211> 300  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> PCR amplification product using Bacillus subtilus as template  
 source and SEQ ID NO:24 and SEQ ID NO:25 as primers  
  
 <400> 25  
 gctttcaagc tgtcaatgcc ggaaaaaaaaa ttgagctttc agtggtttgc gtgggatggc 60  
 tcttcctatg tgcgcatgaa tacgcaaaac tggctgacaa agcaaatctt tttccgtttt 120  
 ttaaaaagta catatttctt caaaggaaaa aagcaaaaga tgtttttagc tgaaggaaaa 180  
 atgaaaacga aagataaaaa cagaggctga aagccatttt taagcgtttt tcttttcttg 240  
 ttgcatcatt tacaatacat acaaccgcaa ggagaggagg aatcgcatgt tcaatcggat 300  
  
 <210> 26  
 <211> 27  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> amyE-1: Forward primer  
  
 <400> 26  
 gctctagacg aattatatgg atgtgac 27  
  
 <210> 27  
 <211> 30  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> amyE-2: Reverse primer  
  
 <400> 27  
 gcctagagct cggatctcct tttccgattg 30  
  
 <210> 28  
 <211> 34  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> Kn-1: Forward primer  
  
 <400> 28  
 caatctgcag tcgcatgag aatagtgaat ggac 34  
  
 <210> 29

<211> 28  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> Kn-2: Reverse primer  
  
 <400> 29  
 cggtacctc aaaatggtat gcgttttg 28  
  
 <210> 30  
 <211> 269  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> artificially made spoVG RBS  
  
 <400> 30  
 gaagatctct cgagggtacc ttgatacact aatgctttta tatagggaaa aggtggtgaa 60  
 ctcatatgaa tcgagcttct agagagctcc catggaacta tgtgattacg aaaatatatc 120  
 cttttccac cacttgagta tacttagctc gaagatctct cgagggtacc ttgatacacc 180  
 taatgctttt atataggga aaggtggtgc atggaactat gtgattacga aaatatatcc 240  
 cttttccacc acttgagtat acttagctc 269  
  
 <210> 31  
 <211> 29  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> spoVG1 primer  
  
 <400> 31  
 gaagatctct cgagggtacc ttgatacac 29  
  
 <210> 32  
 <211> 30  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> spoVG2 primer  
  
 <400> 32  
 ctatataaaa gcattagtgt atcaaggtac 30  
  
 <210> 33  
 <211> 30

<212> DNA  
 <213> unknown  
  
 <220>  
 <223> spoVG3 primer  
  
 <400> 33  
 ctaatgcttt tatataggga aaaggtggtg 30  
  
  
 <210> 34  
 <211> 30  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> spoVG4 primer  
  
 <400> 34  
 ctcgattcat atgagttcac caccttttcc 30  
  
  
 <210> 35  
 <211> 29  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> spoVG5 primer  
  
 <400> 35  
 gactgcagct cgagggtacc ttgatacac 29  
  
  
 <210> 36  
 <211> 41  
 <212> DNA  
 <213> unknown  
  
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 <223> Gntterm-1: Forward primer  
  
 <400> 36  
 gcggatccag gcctaactaa ttaacctgta ttaaaaacac g 41  
  
  
 <210> 37  
 <211> 33  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> Gntterm-2: Reverse primer  
  
 <400> 37  
 gctctagagt taaccttctg ttgtttggga tag 33

<210> 38  
 <211> 40  
 <212> DNA  
 <213> unknown  
  
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 <223> B-toxoid3 primer  
  
 <400> 38  
 aactgcagag atctcatatg aagaaaaaat ttatttcatt 40

<210> 39  
 <211> 32  
 <212> DNA  
 <213> unknown  
  
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 <223> Btoxoid2 primer  
  
 <400> 39  
 cgcggtatcct taaatagctg ttactttgtg ag 32

<210> 40  
 <211> 33  
 <212> DNA  
 <213> unknown  
  
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 <223> rpsF-3 primer  
  
 <400> 40  
 ggaattcctg caggtgactt tgagcggggc ttc 33

<210> 41  
 <211> 24  
 <212> DNA  
 <213> unknown  
  
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 <223> rpsF-4 and rpsF-5 primer  
  
 <400> 41  
 cgtactttct catatgtttg cacc 24

<210> 42  
 <211> 28  
 <212> DNA  
 <213> unknown  
  
 <220>  
 <223> amyEback-1 primer

<400> 42  
cccaagcttt cgacatggat gagcgatg 28

<210> 43  
<211> 28  
<212> DNA  
<213> unknown

<220>  
<223> amyEback-2 primer

<400> 43  
gcagctcgag gctccggcgc aaatgcag 28

<210> 44  
<211> 29  
<212> DNA  
<213> unknown

<220>  
<223> amyEfront-1 primer

<400> 44  
cgcgagctca acaaaattct ccagtcttc 29

<210> 45  
<211> 31  
<212> DNA  
<213> unknown

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<223> amyEfront-2 primer

<400> 45  
cggctctagaa gtttttaatt tgtgtgtttc c 31

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<211> 23  
<212> DNA  
<213> unknown

<220>  
<223> cmR-1 primer

<400> 46  
cggaattct catgtttgac agc 23

<210> 47  
<211> 30  
<212> DNA  
<213> unknown

<220>  
 <223> cmR-2 primer

<400> 47  
 cgcgagctt cccagtagta ggttgaggcc 30

<210> 48  
 <211> 41  
 <212> DNA  
 <213> unknown

<220>  
 <223> Gntterm-3: Forward primer

<400> 48  
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<210> 49  
 <211> 315  
 <212> DNA  
 <213> unknown

<220>  
 <223> upstream region of the rpsF gene, indicating the location of the  
 -10 and -35 regions and AT-rich stretches further upstream, as  
 well as the RBS and the start codon of the rpsF gene

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 ctgagtgtgc aggaattatt catagtgact ttgagcgggg cttcattcgt gctgagacag 60  
 ttgcttatga ggatcttctt gcgggcggcg gtatggcagg agctaaagag gcaggaaaag 120  
 tccgccttga agggaaagaa tatgtggtcc aagacggaga tggtattcat ttccgattta 180  
 atgtatagga tgcagttgta aagggacaag agctttggta taatataaaa ttgtgagtaa 240  
 tagaattatt gctccttgcc cattatgggc cgcttagtcc aaaaggaggt gcaaacagat 300  
 ggaagttact gacgt 315

<210> 50  
 <211> 312  
 <212> DNA  
 <213> unknown

<220>  
 <223> upstream region of the ywpH gene

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 cctatgtgcg catgaatacg caaaactggc tgacaaagca aatctttttc cgttttttta 120  
 aaagtacata tttcttcaaa ggaaaaaagc aaaagatggt ttagctgaa ggaaaaatga 180



aaacgaaaga taaaaacaga ggctgaaagc catTTTTaag cgtttttctt ttcttgttgc	240
atcatttaca atacatacaa ccgcaaggag aggaggaatc gcatgttcaa tcaggtcatg	300
cttgtcggac gt	312